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Effect of isolated bacterial strains from distillery wastewater on power generation in microbial fuel cell

N. Samsudeen, T.K. Radhakrishnan, Manickam Matheswaran*

Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli, India

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ABSTRACT

The effect of isolated bacterial strains on power generation in microbial fuel cell (MFC) using distillery wastewater as a substrate was investigated. The strains were isolated from wastewater and identified using 16S rRNA gene sequences. The isolated species was found to be of *Bacillus* types and the strains were designated as *L. sphaericus* SN-1, *L. sphaericus* SN-2 and *B. safensis* SN-3, respectively. The strains were used as biocatalyst for the generation of electricity and treatment of wastewater in the MFC. The result showed that each strain has different current generation capacity and wastewater treatment efficiency. The MFC inoculated with *L. sphaericus* SN-2 produced a maximum open-circuit voltage (OCV) of 646 ± 5 mV and peak power density of 104 ± 3 mW/m^2 with higher treatment efficiency of 63.4 ± 0.5%. The effect of wastewater pH (6–8), COD concentration (3200, 4800 and 6400 mg/L) and buffer on power generation in the MFC were investigated. Based on the performance of the MFC, *L. sphaericus* SN-2 can be used as a biocatalyst for electricity generation from the distillery wastewater.

1. Introduction

Alcoholic distillery industries release about 8–15 L of wastewater for the production of every liter of alcohol during the process. The distillery wastewater is the most complex substrate which leads to pollution in nearby water bodies when they are discharged into the environment [1,2]. The color of wastewater does not allow penetration of sunlight and thus causes deleterious effects on aquatic life. The treatment of distillery wastewater by various methods such as biodegradation, flocculation, electrochemical and membrane filtration has been investigated [3]. Most of the conventional treatment processes need energy which is not economical for attaining sustainability. The MFC is a novel green technology to generate electricity during the wastewater treatment which supports the combined issues of energy crisis and environmental safety [4,5]. The MFC is a hybrid bioelectrochemical system that directly converts the chemical energy into electricity by the oxidation of organic matter in the presence of microorganisms under ambient conditions. The voltage generation due to the bacterial metabolic activity in the anode and the electron acceptor conditions in the cathode results in bioelectricity [6–8].

The distillery wastewater contains high organic matter that can provide a good source for electricity production in the MFCs and easily biodegradable [9]. The distillery wastewater has been widely examined as a substrate for electricity generation in MFC using mixed microbial community, simultaneously leading to the treatment of the wastewater pollutant [10,11]. Zhang et al. [12] and Huang et al. [13] have developed a coupled system of MFC technology with conventional anaerobic/aerobic processes such as up-flow anaerobic sludge blanket reactor–microbial fuel cell–biological aerated filter (UASB–MFC–BAF) and anaerobic fluidized bed–microbial fuel cell (AFB–MFC). This system showed the feasibility of electricity generation with the simultaneous removal of pollutants from the molasses wastewater. Mohanakrishna et al. [14] reported that the MFC could effectively reduce chemical oxygen demand (COD), color and dissolved oxygen content of the distillery wastewater. Various unit operations pertaining to wastewater treatment such as biological treatment (anaerobic), electrolytic dissociation and electrochemical oxidation can be performed in the MFC.

The performance of MFC was affected by various intrinsic and extrinsic factors such as types of substrate and its concentration, solution pH, electrode materials, system architecture, temperature and types of microorganism during wastewater treatment [15–17]. The presence of a microorganism as a biocatalyst either in pure strain or mixed community plays a crucial role in the generation of electricity through metabolic reaction during the removal of

* Corresponding author.
E-mail address: math_chem95@rediffmail.com (M. Matheswaran).

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pollutants from the wastewater. Many of the researchers evaluated the performance of MFC in terms of electricity generation and treatment of wastewater by inoculating with the mixed microbial communities [18,19]. Nevertheless, roles of the individual microorganism and the mechanisms involved contribute to power generation, and understanding the treatment efficiency becomes difficult using mixed microbial community as a biocatalyst. The pure bacterial strains including g*ram-positive Corynebacterium* sp., *B. subtillis, E. cloacae* etc. have been used to investigate the extent of power generation and utilization of wastewater from various sources in the MFC [20–22]. The exoelectrogenic activities of bacterial strains were altered with pH conditions in the MFC. Liu et al. [20] reported that the higher electrochemical activity of strain g*ram-positive Corynebacterium* sp. MFC03 at pH 9 as compared with pH 8. Kim et al. [23] studied the performance of MFC using low-pH distillery wastewater as a substrate without pH control under continuous mode. They reported *Caldiserica* might significantly contribute to electricity generation of single chamber MFC using low-pH wastewater and high-R_{ext}. Ganapathy et al. [24] reported that the isolated strain of *Nostoc muscorum,* belonging to the phylum *Cyanobacteria* was found in the distillery wastewater which could be potentially used for the biological treatment. Ha et al. [25] used uncultured *Bacteroidetes bacterium* obtained from thermophilic anaerobic digestion sludge for the treatment of distillery wastewater in the MFC.

In this study, the feasibility of using inherent microbes isolated from the distillery wastewater was tested in a dual chamber MFC for power generation along with treatment. The isolated strains were identified by 16S rRNA gene sequencing based on nucleotide homology and phylogenetic analysis. The electrochemical activities of each strain were investigated by cyclic voltammetry (CV) and compared with those of mixed culture. The effect of wastewater inoculated with isolated strains on power generation, COD removal and columbic efficiency in the MFC was studied. The performance of MFC was investigated under different pH conditions, wastewater COD concentration and also compared in the presence and absence of phosphate buffer.

2. Materials and methods

2.1. Wastewater characteristics

The wastewater was used as a substrate that collected nearby distillery industries Trichy, India. The important characteristics of the distillery wastewater were: pH: 4.9 ± 0.05, COD: 80,000–90,000 mg/L, TDS: 18460–20200 mg/L, conductivity: 33.30 ±3 mS/cm and color – dark brown, odor – burnt sugar. The pH, conductivity and TDS of wastewater were determined using multiparameter analyzer (CyberScan PC650, Eutech's). The collected wastewater was stored in refrigerator at 4 ± 1 °C prior to use.

2.2. Isolation of bacteria

Organisms present in the distillery wastewater were isolated using a liquid nutrient medium. The raw distillery wastewater (DW) was diluted into 25 times using deionized water for isolating the bacteria. The bacterial strains were isolated from the diluted DW using serial dilution followed by pour plate and streak plate techniques. The nutrient agar was prepared using peptic digest (5 g/L), Beef extract (1.5 g/L), sodium chloride (5 g/L), yeast extract (1.5 g/L) and 15 g agar for 1000 mL medium. The prepared agar media was sterilized in an autoclave at 121 ± 2 °C for 15 mins. The distillery wastewater of 0.1 mL was spread on the nutrient agar plates and incubated at 37 °C for 24 h. The isolated strain was repeatedly subcultured for obtaining a pure culture. After incubation, bacterial colonies were selected and subjected to preliminary screening, phenotypic and genotypic characterization.

2.3. Identification of bacteria

For identification of bacteria, overnight cultures of each isolate in nutrient broth were considered. All isolates were initially tested for gram reaction and subjected to biochemical test. The genomic DNA was extracted using a modified phenol–chloroform–isoamyl alcohol method. For genotypic identification, the bacterial 16S rDNA fragments in genomic DNA were amplified using universal 16S rDNA primers, forward primer 8F (5′AGA GTT TGA TCC TGG CTC AG −3′) and reverse primer 1492R (5′ CGG TTA CCT TGT TAC GAC TT-3′). PCR amplification was conducted in an automated thermal cycler (BIORAD T100TM) using the following protocol. The final reaction PCR mixture contained 1 μL of DNA, 400 ng of reverse primer, 4 μL of deoxynucleotide triphosphate mixture, 10 μL of Taq polymerase buffer and 1 μL of Taq polymerase. The amplification condition was 94 °C for 5 min, followed by 35 cycles of denaturation at 92 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min. A final extension step was carried out at 72 °C for 5 min. The PCR products were analyzed by 1.4% (W/V) agarose gel electrophoresis with ethidium bromide (0.5 g/mL) for 1 h and visualized on a UV transilluminator. The PCR products were purified and quantified photo metrically (Model UV-1700 Shimadzu). The 16S rDNA sequence was aligned and identified by GenBank using the BLAST program. The closest known relatives of the isolates were determined by performing a sequence database search. Distance matrix was generated using the Ribosomal Database Project RDP database and the phylogenetic tree was constructed using MEGA 4 [26–29].

2.4. MFC configuration and operation

Four identical MFCs were constructed using a non-conducting polymeric material (Plexi glass) with working volume of 210 mL in 230 mL of the total volume. The MFC consists of an anode and a cathode compartment separated by a proton exchange membrane (PEM) (Nafion^{TM} 117, Dupont Co,). The membrane was pretreated using 0.5 M H_2SO_4 followed by 5% H_2O_2 at 70–80 °C for 1 h before used. The anode and cathode electrodes were made of graphite plates with the dimension of 4 cm × 6 cm × 0.3 cm. The electrodes were placed at a distance of 1 cm on either sides of the membrane. Prior to use, the electrodes were soaked in deionized water for 24 h. Copper wires were used to provide connection between the anode and cathode electrodes.

The anode and cathode compartments were filled with sterilized distillery wastewater and potassium ferricyanide (50 mM) in phosphate buffer (50 mM) respectively. The isolated liquid broth of 5 mL was added in the anode compartment. The anodic solution pH was adjusted using orthophosphoric acid (88%) and 3 N NaOH with respect to the experimental conditions. The cathodic solution pH was adjusted to 7.5. The sterilized wastewater was replaced once the cell voltage started to drop under the open-circuit mode. In order to maintain the anaerobic environment, the anode compartment was completely sealed and then purged with N_2 gas for 10 min at every feeding event. All the experiments were carried out inside the laminar flow cabinet to avoid the cross-contamination of microorganisms. The system was operated in batch mode under ambient conditions (31 ± 2 °C and 1 atm). The MFC was operated under OCV mode for several cycles (8 days per cycle) to obtain the stable potential generation. The polarization, coulombic efficiency and COD removal efficiency were measured after attaining the stable potential. The effect of MFC performance was investigated by varying COD concentration (3200, 4800 and 6400 mg/L) and pH